Multiple evidences of past evolution are hidden in nrDNA of *Juniperus arizonica* and *J. coahuilensis* populations in the trans-Pecos, Texas region

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ABSTRACT

Geographical analysis of variation in nrDNA polymorphisms of *J. arizonica* and *J. coahuilensis* in the trans-Pecos, TX region showed multiple patterns of hybridization, both modern and relictual (Pleistocene) introgression, incomplete lineage sorting and relictual hybridization. The concept that nrDNA from a single plant could harbor multiple evidences of past evolution appears to be novel. Total nrDNA polymorphisms were maximal in the Ft. Davis, Alpine, Marfa trans-Pecos area and on the granitic rocks at Hueco Tanks State Park, TX. Published on-line www.phytologia.org *Phytologia 99(1): 38-47 (Jan 19, 2017)*. ISSN 030319430.

KEY WORDS: *Juniperus arizonica, J. coahuilensis,* Cupressaceae, hybridization, introgression, incomplete lineage sorting, nrDNA polymorphisms, petN-psbM DNA.

Recently, Adams (2016) found (by petN-psbM sequencing) that *Juniperus arizonica*, previously known only from Arizona and New Mexico, occurs in trans-Pecos Texas in the Franklin Mtns., Hueco Mtns., Hueco Tanks State Park, Quitman Mtns., Eagle Mtns. and Sierra Vieja Mtns., primarily on igneous material (Figs. 1, 2). These trans-Pecos juniper populations have previously been identified as *J. coahuilensis* . These taxa have very distinct differences in their DNA and are in separate clades (Adams, 2014, Adams and Schwarzbach, 2011, 2013). The cp region petN-psbM is especially efficient in separating these taxa, as 5 SNPs occur in the 794 bp region.

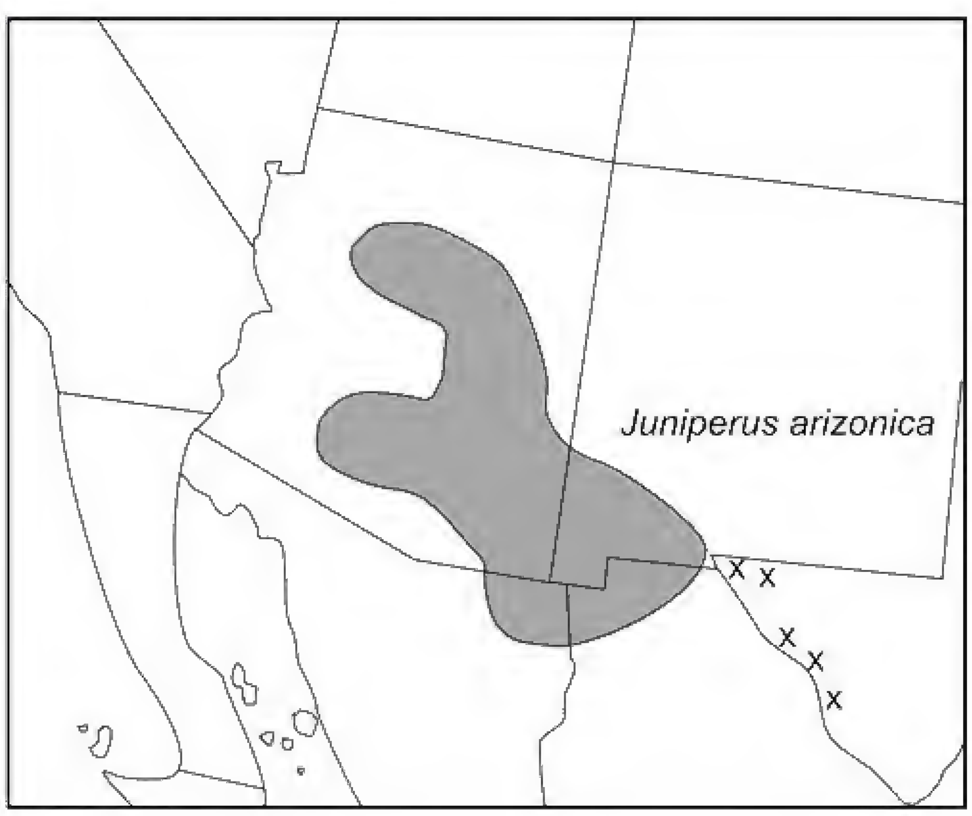


Figure 1. Distribution of *J. arizonica* (Adams 2016).

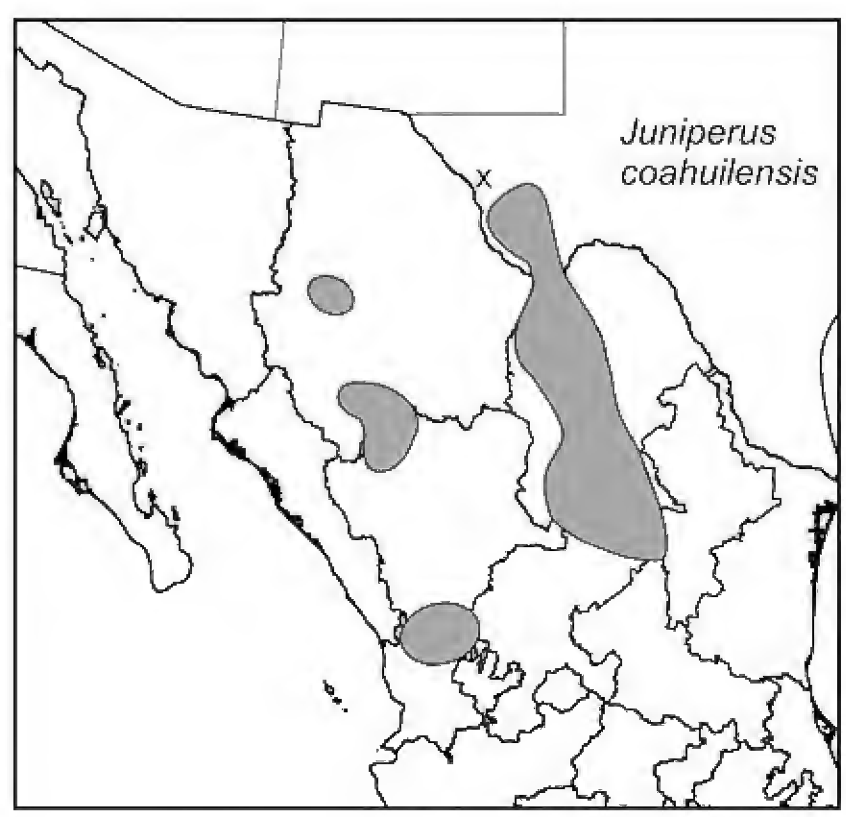


Figure 2. Distribution of *J. coahuilensis*

Detailed mapping of plants by their cp DNA (petN-psbM) showed that all the plants (or specimens) in New Mexico and northern Mexico, as well as plants examined from the Franklin Mts., Hueco Tanks SP, Quitman Mts., Eagle Mts., and one plant from Sierra Vieja Mtns. contained the *J. arizonica* cp DNA (Fig. 3). Junipers from Ft. Davis, Alpine, Marfa and Big Bend (1) all had the *J. coahuilensis* cp DNA (Fig. 3). The occurrence and extent of hybridization and introgression in that region is not known, except for a study of hybridization between *J. coahuilensis* and *J. pinchotii* in the Chisos Mtns. (Adams and Kistler, 1991).

Recently, Adams et al. (2016) have reported that in the sister genus, *Hesperocyparis*, artificial hybrids between *Hesperocyparis* (= *Cupressus* in part) *arizonica* and *H. macrocarpa*, nrDNA was inherited as heterozygous for diagnostically different sites. They concluded that, at least in *Hesperocyparis* (and likely in the Cupressaceae, including *Juniperus*), analysis of heterozygous nrDNA (ITS) could be used for the detection and analysis of hybridization. Because F_2 progeny and backcrosses were not analyzed, they could not comment on the amount and/ or speed of lineage sorting in *Hesperocyparis*.

The purpose of this paper is to report on the composition of nrDNA in populations in the trans-Pecos, TX region and the investigation of hybridization, introgression and incomplete lineage sorting.

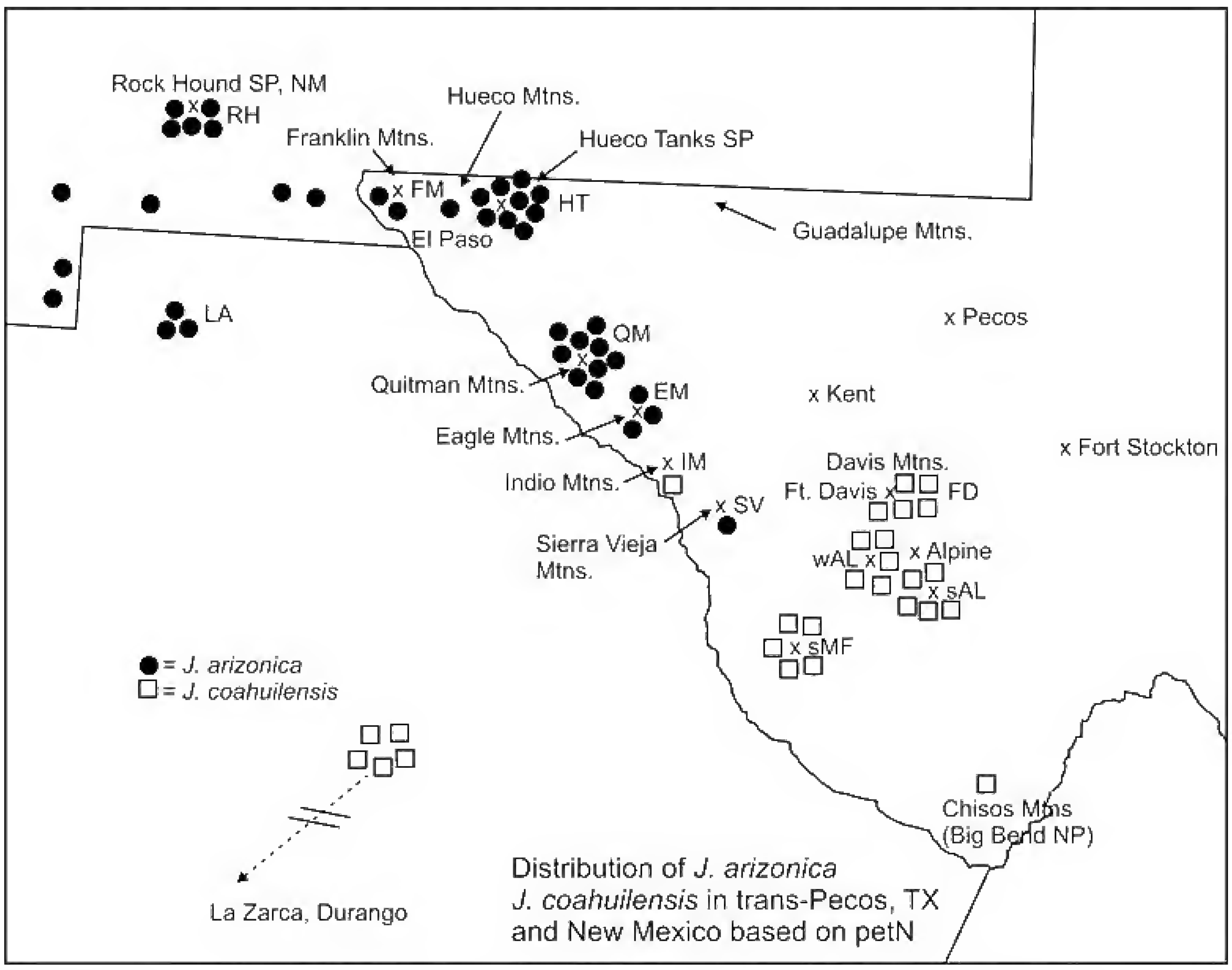


Figure 3. Distribution of *J. arizonica* and *J. coahuilensis* based on petN-psbM cp data.

MATERIALS AND METHODS

Plant material and populations studied:

Sedona, AZ

J. arizonica by petN DNA, common in grassland. tree 6 m tall. female, with *J. osteosperma* on alluvial soil. On AZ highway 179, between Sedona and I17. 34° 42.431'N, 111° 46.369' W, 1150 m, 13 March, 2005, Yavapai Co., AZ, *Robert P. Adams 10634-10636*,

Cottonwood, AZ

J. arizonica by petN DNA, abundant, on alluvial fan, 3 mi. SW of Cottonwood, AZ, on D. Thornburg's property, 34° 41' 17.4" N, 112° 03' 05.46" W, 4060 ft., 13 Jan., 2010, Yavapai, Co., AZ, Coll. David Thornburg ns, Lab Acc. *Robert P. Adams 14908-14913*,

Southern New Mexico:

J. arizonica by petN DNA, Hidalgo Co., NM, Animas Mtns, 31.61176° N, 108.7791° W, 5750', Seinet Cat # 57778, *Wagner 1283*, 22 Jul 1975, Lab Acc. *Robert P. Adams 14697*,

J. arizonica by petN DNA, Luna Co., NM, Tres Hermanos Mtns, 31.9010° N, 107.7794° W, 4250', Seinet Cat # 85666, *J L Carter 1246*, 14 Aug 1993, Lab Acc. *Robert P. Adams 14698,*

J. arizonica by petN DNA, Hidalgo Co., NM. Animas Mtns, 31.5938° N, 108.7684° W, 6000', Seinet Cat # 57776, *Wagner 1005*, 17 Jun 1975, Lab Acc. *Robert P. Adams 14701,*

J. arizonica by petN DNA, no cones, Hidalgo Co., NM, Animas Peak, Animas Mtns., 31.5813° N, 108.7843° W, 8452' (Google Earth), Seinet Cat # 25131, *WC Martin 4678*, 29 Oct 1960, Lab Acc. *Robert P. Adams 14705*,

J. arizonica by petN DNA, Hidalgo Co., NM, Big Hatchet Mtns.,with Quercus, Parthenium, Ocotillo, Mesquite, Agave 31.6249° N, 108.36425° W, 5350', *Ken Heil 9254*, 28 May 2010, Lab Acc. *Robert P. Adams 14716*,

J. arizonica by petN DNA, Grant Co., NM, ca 1.5 mi. s of NM hwy 9, near 'Old Hachiti' townsite. Chihuahuan desert scrub - creosote, Lycium koberlina and Dalea formosa. 31.9139° N, 108.41472° W, 4745', *Ken Heil 32357*, 29 Apr 2010, Lab Acc. *Robert P. Adams 14717*,

Rock Hound State Park, NM (type locality, *J. arizonica*)

J. arizonica by petN DNA, multi-stemmed shrubs to 4m, in Bouteloua grassland. Pollen shed in Mar-April?, Fruit rose color. Rock Hound State Park. 17km S, and 8 south of Deming, NM, 32° 11.161'N, 107° 36.651' W, 1420 m, 6 Feb., 1996, Luna Co., NM,, *Robert P. Adams 7635-7637*

J. arizonica by petN DNA, common in *Bouteloua* grassland. shrub-trees to 3-5 m., Rock Hound State Park., 32° 11.161'N, 107° 36.651' W, 1420 m, 12 Mar, 2005, Luna Co., NM, *Robert P. Adams 10630*,

Quitman Mtns.

J. arizonica by petN, Hudspeth Co., TX, common on degraded granite, north face of Quitman Mtns., with desert-scrub. On south side of I10, ~6.3 mi. w of Sierra Blanca, TX, 31°12' 25" N; 105° 27' 51" W, 4629', *Robert P. Adams 14798-14806,* 12 March 2016,

Hueco Tanks St. Park, TX

J. arizonica by petN El Paso Co., TX, uncommon, 50- 100 trees seen, on granite, Hueco Tanks St. Park, 31° 54' 49.7" N; 106° 02' 6.8" W, 4560', *Robert P. Adams 14827-14835*, 18 March 2016. *Robert P. Adams 14718.*

11.2 s of Alpine, TX on Tex 118

J. coahuilensis, by petN, Brewster Co, TX, abundant in grassland, 11.2 s of Alpine, TX on Tex 118. 30° 14' 08" N; 103° 34' 00" W, 5222', *Robert P. Adams 14807-14811,* 15 March 2016,

11.0 mi w of Alpine, TX on US 90

J. coahuilensis, by petN, Brewster Co, TX, 11.0 mi w of Alpine on US 90, abundant in grassland, in Paisano Mtns., 30° 17' 42" N; 103° 48' 02" W, 4967', *Robert P. Adams 14812-14816,* 15 March 2016,

4.2 mi se of Ft. Davis, TX on Tex 118, CDRI

J. coahuilensis, Jeff Davis Co., TX, common locally, in grassland. 4.2 mi se of Ft. Davis, on Tex 118, e 1.0 mi into Chi. Desert Res. Inst., 39° 09' 27.54" N; 86° 18' 23.31" W, 5050', *Robert P. Adams 14817-14821*, 16 March 2016,

19.4 mi. s of Marfa, TX on US 67

J. coahuilensis, by petN, Presidio Co., TX, common in grassland, 19.4 mi. s of Marfa, on US 67, 30° 04' 07" N; 104° 10' 19" W, 5137', *Robert P. Adams 14822-14826*, 16 March 2016,

La Zarca, Mexico

J. coahuilensis, large population with thousands of trees. 85 km N. of La Zarca on Mex. 45, 1740m, 10 Dec, 1991, Durango, Mexico, *Robert P. Adams 6829-6831*,

Voucher specimens for new collections are deposited in the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM), D (maldehy) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the petN-psbM primers utilized.

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.).

RESULTS AND DISCUSSION

Sequencing petN-psbM yielded 794 bp with 5 SNPs separating *J. arizonica* and *J. coahuilensis.* In addition, nrDNA was sequenced yielding 1270 bp with only 1 SNP (at site 533) separating *J. arizonica* and *J. coahuilensis.* Using these data, samples were classified accordingly (Table 1). Based on heterozygous peaks at site 533, 11 samples were classified as hybrids (AxC, Table 1). According to nrDNA, hybrids occur mostly in the Animas Mts., NM, Hueco Tanks SP, TX and Quitman Mtns., TX (Fig. 4.). Note one hybrid in the Marfa, TX population. The nrDNA data, indicates that populations of *J. coahuilensis* in the Alpine - Ft. Davis - Marfa area are nearly pure. It should be noted that the soils of Hueco Tanks and Quitman Mtns. are granitic, whereas the Alpine - Ft. Davis - Marfa area soils are volcanic.

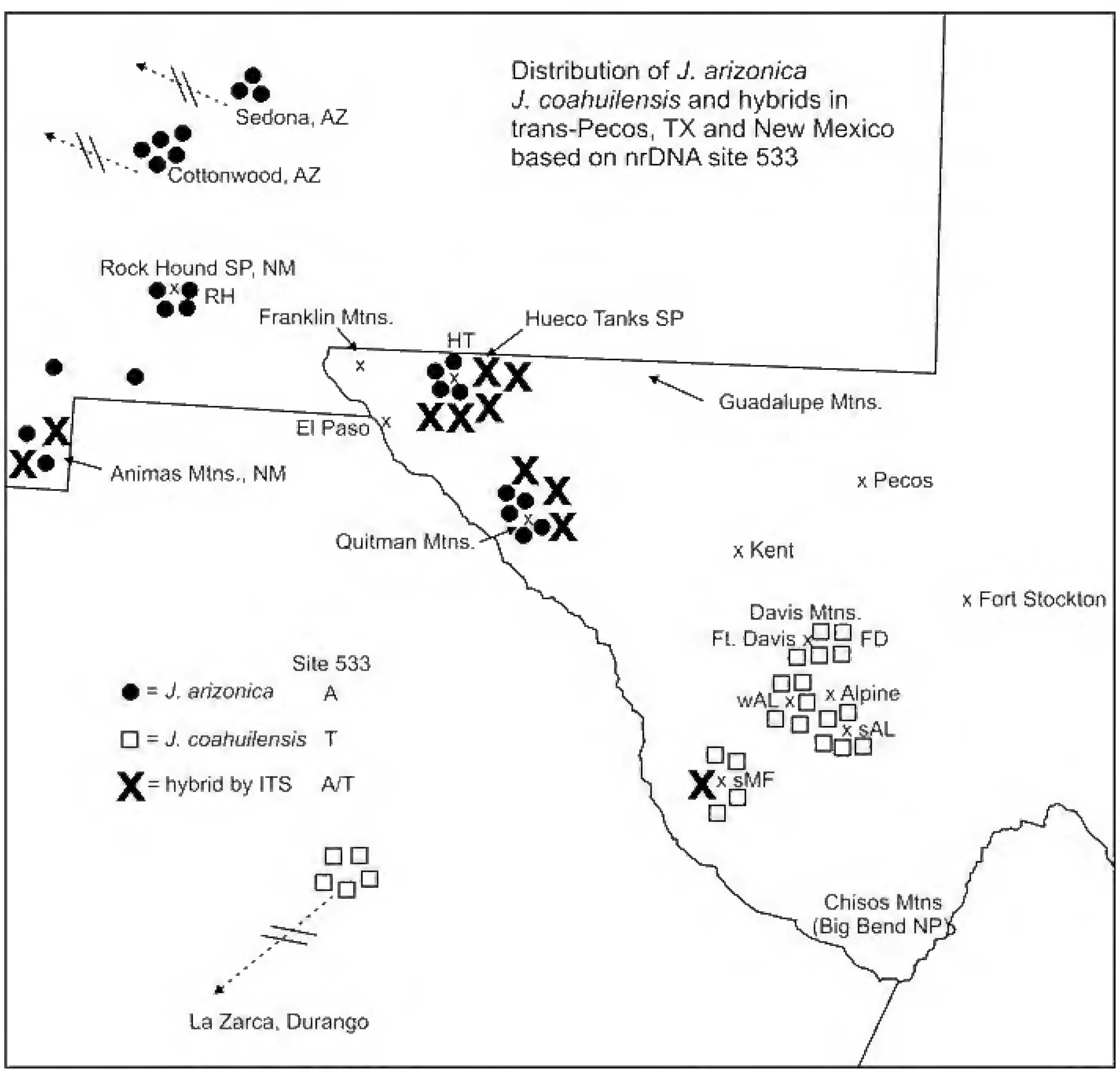


Fig. 4. Distribution of *J. arizonica* x *J. coahuilensis* hybrids based on nrDNA.

In order to visualize the correlation of nrDNA and cp (petN) classifications, each plant was scored for species or hybrid for nrDNA and cpDNA. Mapping this classification shows a relatively sharp demarcation between *J. arizonica* and *J. coahuilensis* (Fig. 5). The zone of hybridization is in Hueco Tanks State Park, Quitman Mtns., and Anima Mtns. and this appears to be a region of introgression northward from *J. coahuilensis* (Fig. 5).

The Hueco Tanks State Park, Quitman Mtns., and Anima Mtns. populations are on granitic soil and the *J. coahuilensis* populations in the Ft. Davis, Alpine, Marfa region are on volcanic soil. Soil differences may be the factor that determines the northern range of *J. coahuilensis* and could present a barrier for additional introgression northward into *J. arizonica*.

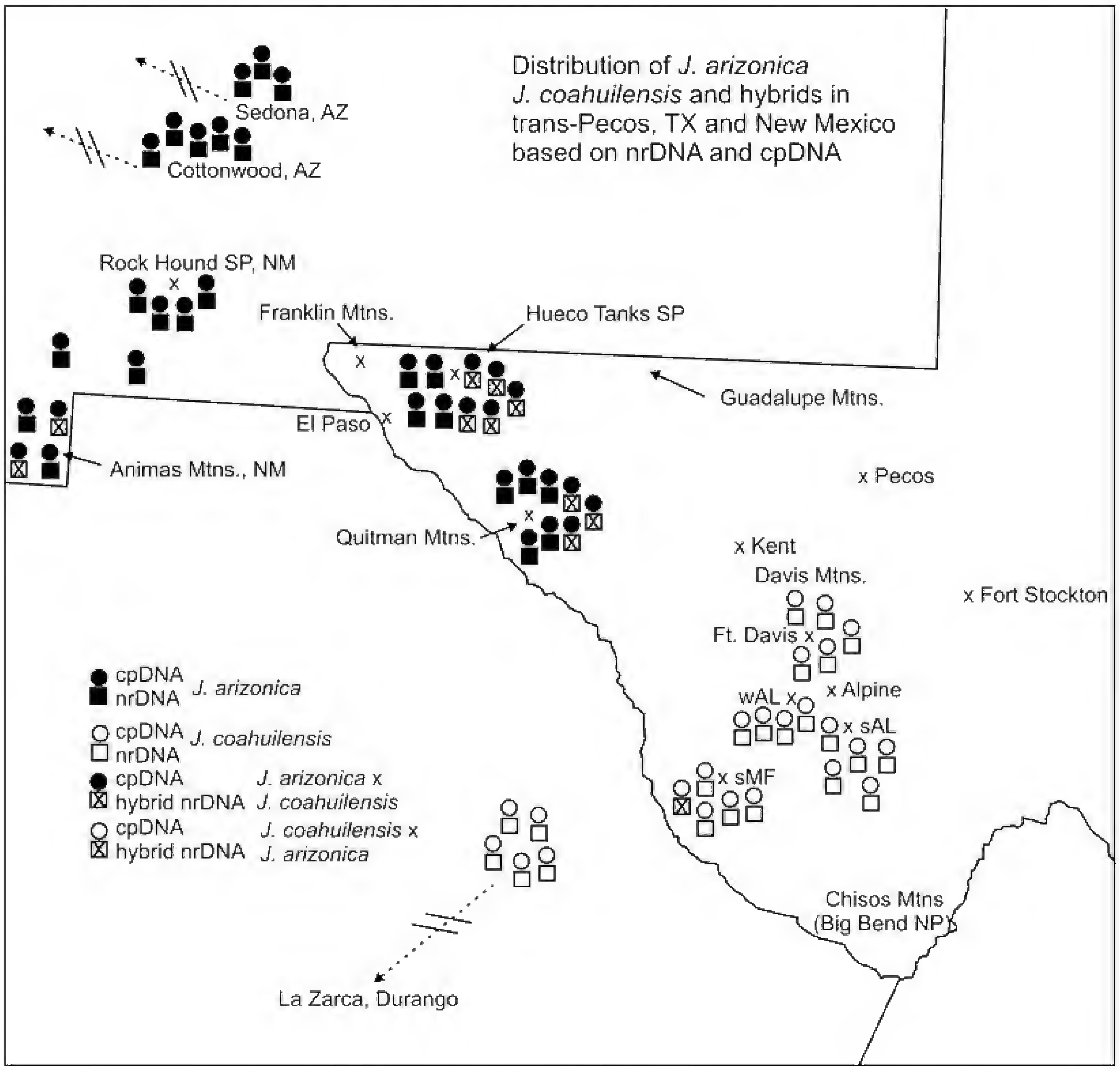


Figure 5. Mapping plants showing their classification as *J. arizonica, J. coahuilensis*, or hybrids for both nrDNA and cpDNA.

Mapping the number of nrDNA polymorphic sites per plant shows very low polymorphic sites in the normal range of *J. arizonica* (NM and AZ, Fig. 6). However, where *J. arizonica* and *J. coahuilensis* hybridize and thence southward, there are several populations with plants having 1 to 6 polymorphic sites (excluding site 533). Hueco Tanks is very variable: 3 plants with 0 polymorphisms; 3 with 1; 2 with 5; and 1 with 6 polymorphisms (Fig. 6). The Davis Mtns - Alpine area is also a region with lots of polymorphisms (Fig. 6). In contrast to the more mountainous sites, the Marfa population (19.3 mi sw of Marfa, in a *Bouteloua* grassland) had low polymorphisms in its nrDNA.

The trans-Pecos region likely experienced a mixing of southern Rockies flora to move southward and the flora of the Sierra Madre Oriental flora to move northward during cooling and heating eras in the Pleistocene. This provided opportunities for many *Juniperus* species, now spatially separated, to hybridize in the past.

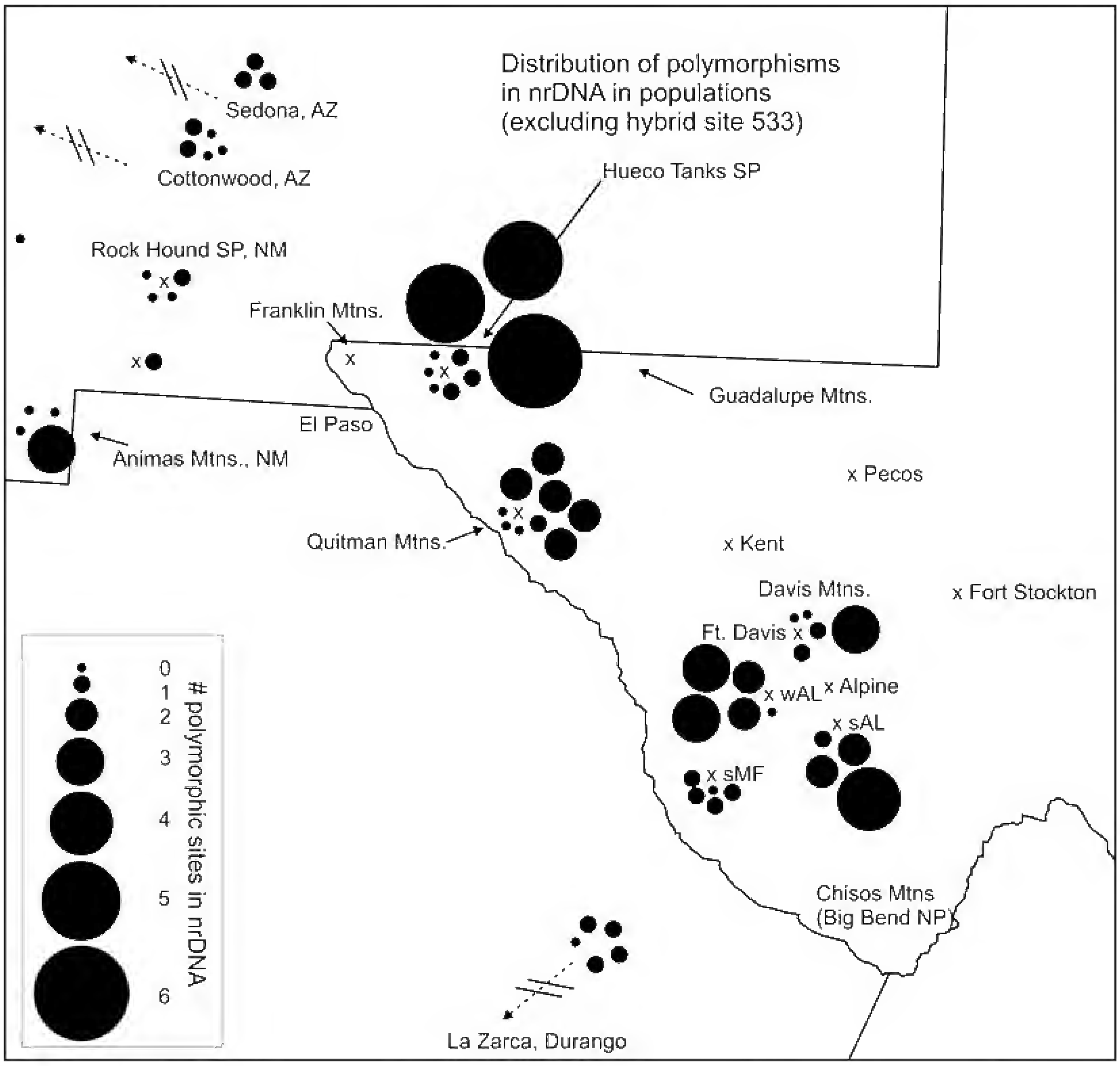


Fig. 6. Distribution of the number of nrDNA polymorphisms/ plant.

A closer examination of individual plant nrDNA site polymorphisms revealed that nrDNA harbors several evolutionary patterns that vary by region. For site 543, 11 plants contained (C/G) and these range from the Quitman Mtns., northwest to Cottonwood and Sedona, AZ (Fig. 7). Only one plant was G/G, and that was in the Cottonwood, AZ population. This is near the northwestern limit of *J. arizonica*. Site 543 might be an indicator of introgression from *J. coahuilensis* into *J. arizonica*.

In addition, another polymorphism occurs (C/T, Fig. 7), but only in the La Zarca, MX population. Additional research is needed to determine if the T comes from hybridization with another Mexican juniper, from incomplete lineage sorting or just a local mutation.

The distribution of variation in site 173 (A/G) is centered between *J. arizonica* and *J. coahuilensis* in the Animas Mtns., NM, Hueco Tanks SP, and Quitman Mtns. (Fig. 8.) This may to be either relictual hybridization, or incomplete lineage sorting.

nrDNA site 304 contains two geographical patterns. One (A/T, Fig. 9) is similar to that for site 173 (Fig. 8) in the Animas Mtns., NM, Hueco Tanks SP, and Quitman Mtns. The second pattern (C/T, Fig. 9) is found in only the south Alpine, TX population. The C/T site might be due to introgression from the east or south from Mexico, perhaps from mixing of taxa during the Pleistocene. Or it may be just a local mutation in that population.

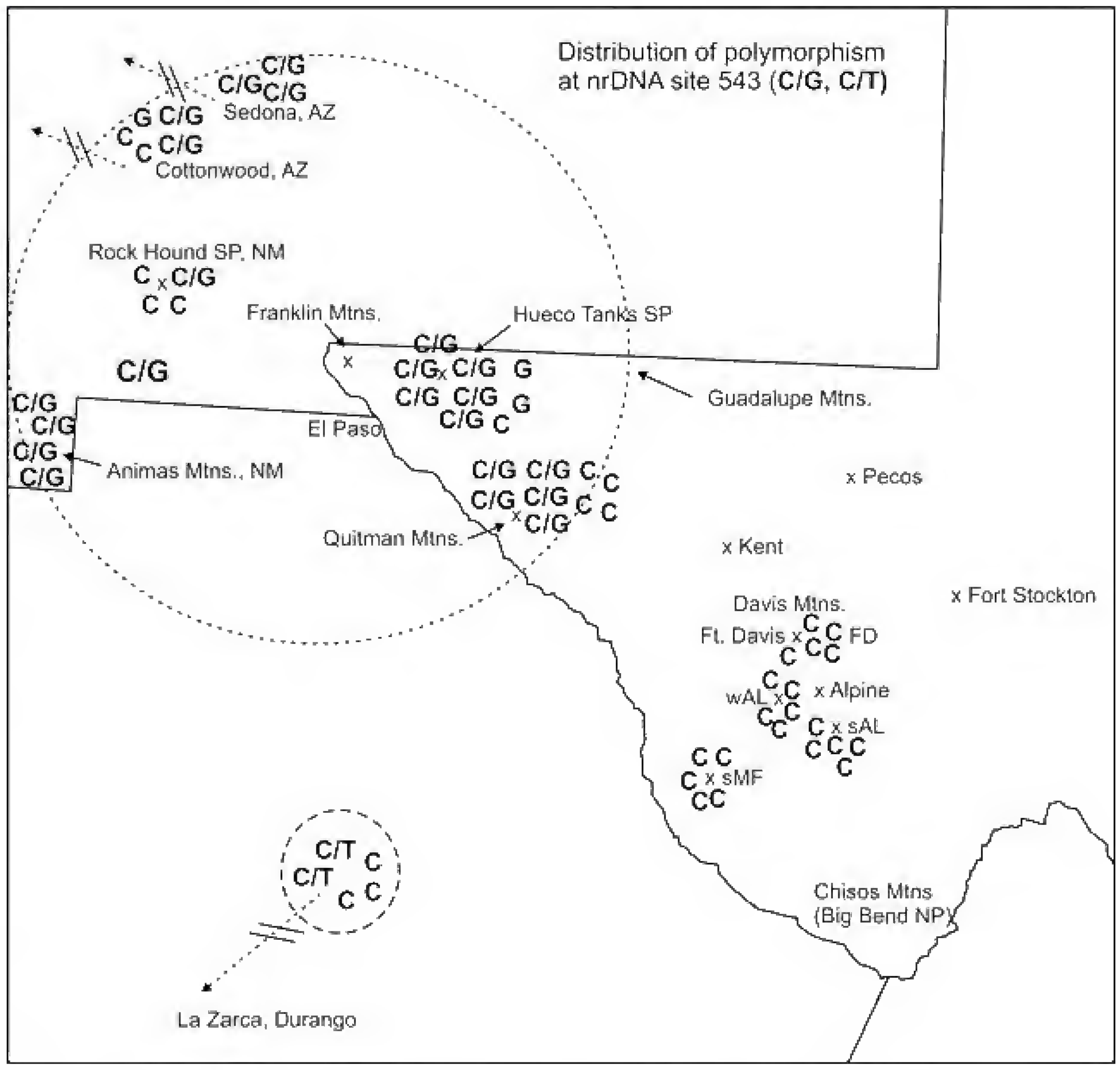
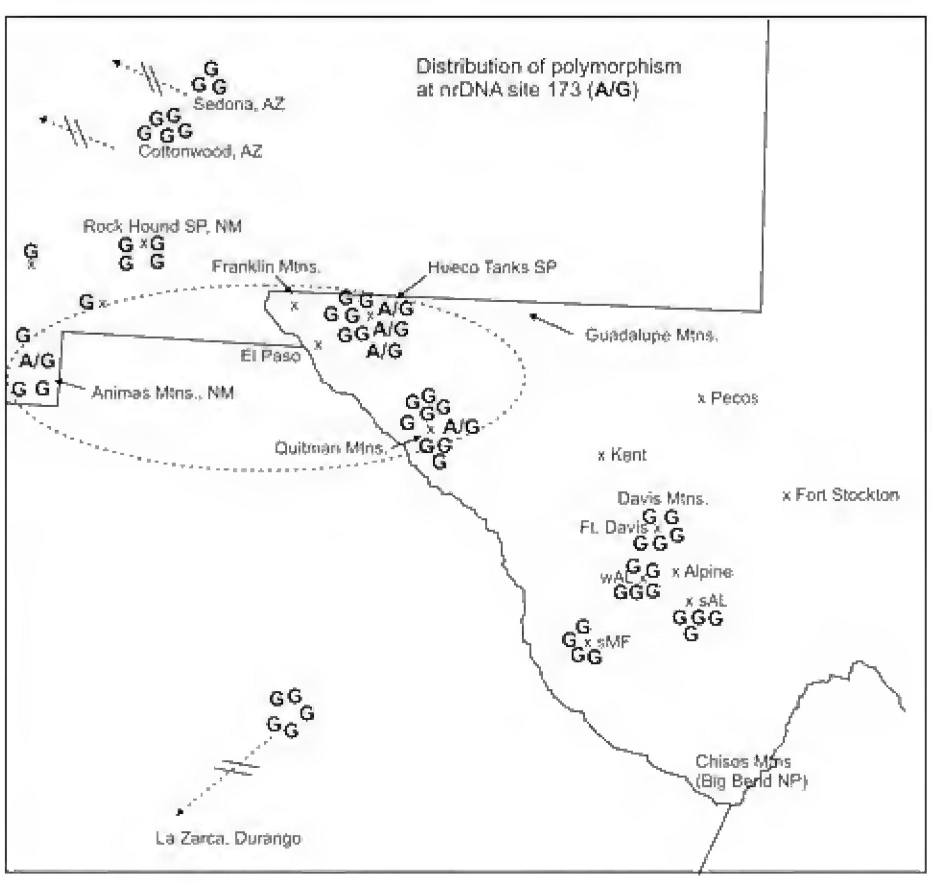


Figure. 7. Distribution of polymorphisms at nrDNA site 543.



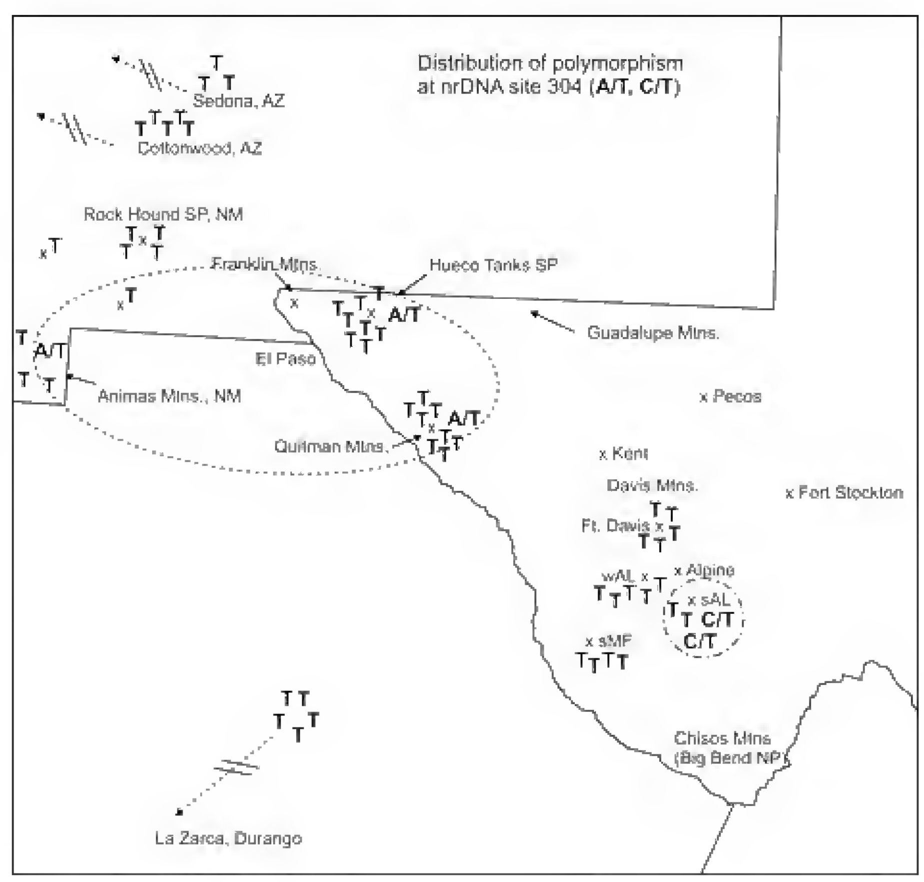


Figure 8. Dist. of nrDNA site 173 polymorphism. Figure 9. Dist. of nrDNA site 302 variation.

Variation in site 318 (C/T, Fig. 10) spans the *J. arizonica - J. coahuilensis* range junction and seems likely to be from relictual hybridization. There appears no source of the C allele in any population of *J. arizonica or J. coahuilensis* examined. Alternatively, it could be incomplete lineage sorting.

Finally, two sites show very similar patterns: both sets of polymorphisms are confined to the Ft. Davis - Alpine - Marfa area and both sites have plants with mixed bases as well as plants with homozygous bases. Site 302 (A/G) was found in all four populations, plants homozygous for A are in all 4 populations, but only one plant homozygous for G was found (in the Ft. Davis population, Fig. 11).

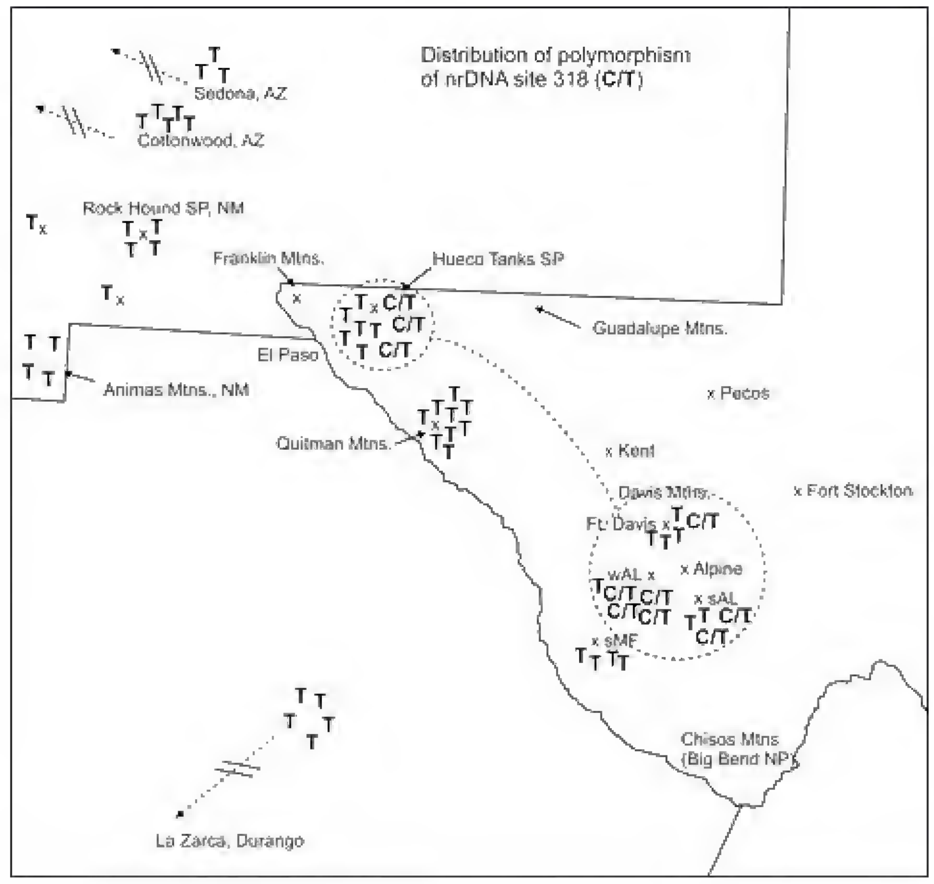


Figure 10. Distribution of site 318 (C/T) polymorphisms.

A similar pattern was found for site 303 (C/T). C/T was present in all four populations, plants homozygous for C were in all 4 populations, but only 2 plants homozygous for T occurred in the Ft. Davis and Marfa populations (Fig. 12). These two sites are difficult to explain. It almost appears that an unknown (to the author) species is present that has (G,C) at 302, 303 and is hybridizing with *J. coahuilensis* (A, C) at 302,303. Other juniper species in the area are *J. pinchotii* (Kent, and Fort Stockton), *J. monosperma* (near Kent), and *J. deppeana* (higher elevations in the area). Of course, it might be Pleistocene relictual hybridization with a species (or its ancestor) now growing in Mexico.

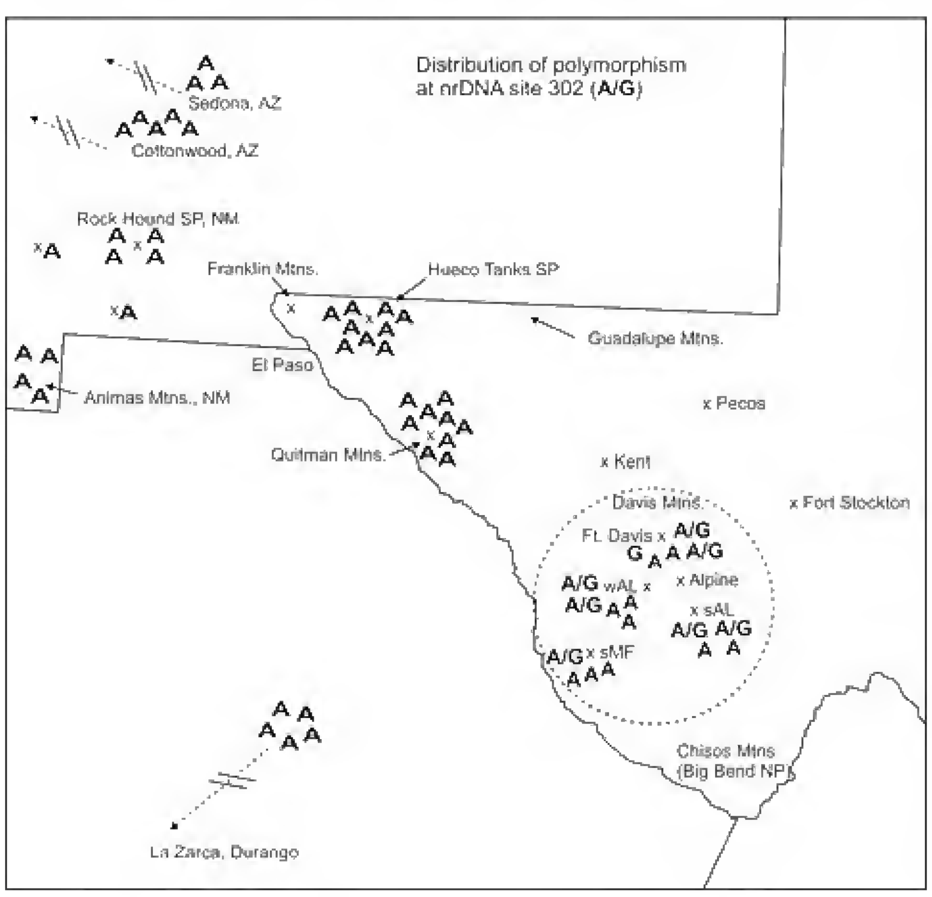


Figure 11. Geographical variation in variation at nrDNA site 302.

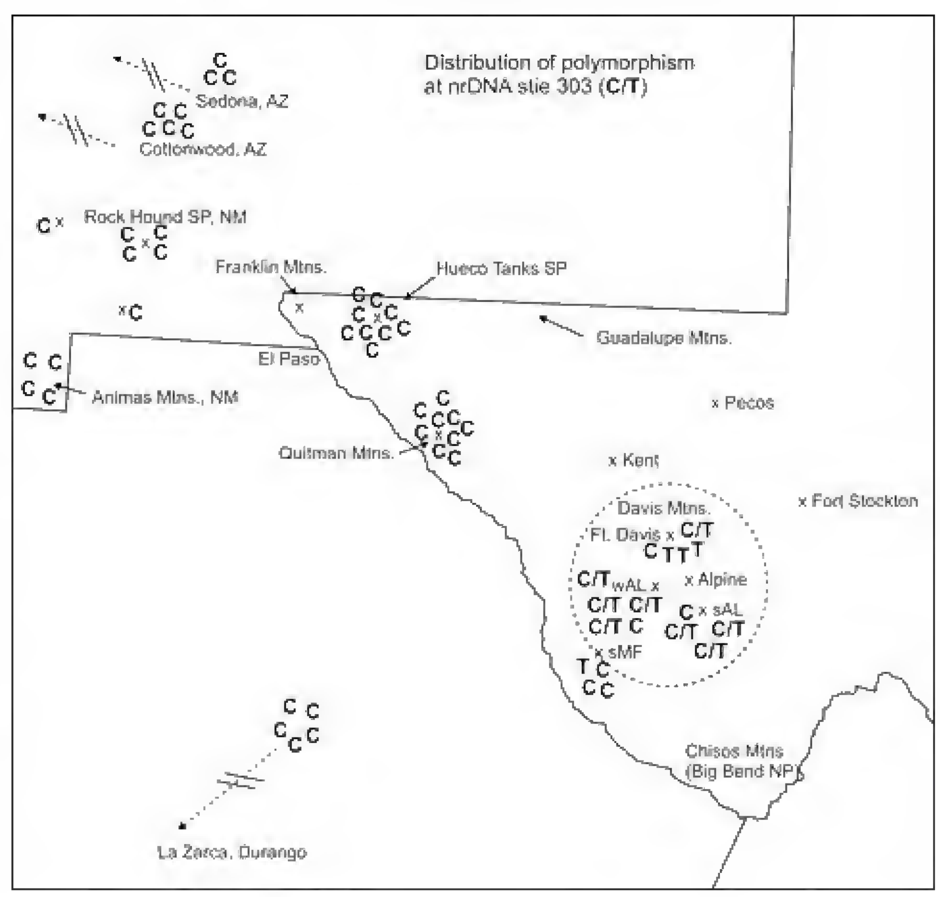


Figure 12. Variation in nrDNA site 303.

SUMMARY

Geographical analysis of variation in nrDNA polymorphisms of *J. arizonica* and *J. coahuilensis* in the trans-Pecos, TX region showed multiple patterns of hybridization, both modern and relictual (Pleistocene) introgression, incomplete lineage sorting and relictual hybridization.

The concept that nrDNA from a single plant could harbor multiple evidences of past evolution appears to be novel. The pre-occupation of evolutionary systematists with phylogeny has resulted a lack of critical variation in nrDNA. Heretofore, the standard procedure is to sequence nrDNA (as the sole proxy of the nuclear DNA), then add in a few cpDNA sequences, then ran the data in a phylogenetics software and publish 'the Phylogeny', and then move on to another genus. That may satisfy a need for a broad evolutionary framework of a group (genus). But, as shown in this report, there may be considerable evidence of past evolutionary events in nrDNA that would be completely ignored (and missed) by only running a phylogenetic analysis.

Total nrDNA polymorphisms were maximal in the Ft. Davis, Alpine, Marfa trans-Pecos area and on the granitic rocks at Hueco Tanks State Park, TX. Additional research using Single Copy Nuclear Genes (SCNG) is needed to further address the variation found in this region.

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Table 1. Classification of samples based on petN and nrDNA. **Bold** are putative hybrids between *J. arizonica* and *J. coahuilensis* by ITS site **533**, A in *arizonica*, T in *coahuilensis*, (A/T in 533) were scored as hybrids (AxC). The more common polymorphic sites are shown. A few rarer, polymorphic sites are listed next to the sample name.

Sample	petN	ITS	173	191	196	302	303	304	318	**533**	543	605	708	# poly
az10634Sedona 181M	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	A	A	1
az10635Sedona 681M	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	A	A	1
az10636Sedona	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	A	A	1
az14908Cottonwood	ariz	ariz	G	G	T	A	C	T	T	A	**G**	A	A	0
az14909Cottonwood	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	A	A	1
az14910Cottonwood	ariz	ariz	G	G	T	A	C	T	T	A	C	A	A	0
az14912Cottonwood	ariz	ariz	G	G	T	A	C	T	T	A	C	A	A	0
az14913Cottonwood 121Y	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	A	A	1
az14717GrantCoNM 181M	ariz	ariz	G	G	T	A	C	T	T	A	C	A	A	0
az7635RockHoundSP	ariz	ariz	G	G	T	A	C	T	T	A	C	A	A	0
az7636RockHoundSP	ariz	ariz	G	G	T	A	C	T	T	A	C	A	A	0
az7637RockHoundSP	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	A	A	1
az10630RockHoundSP	ariz	ariz	G	G	T	A	C	T	T	A	C	A	A	0
az14698LunaCoNM	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	A	A	1
az14697HidalgoCoNM	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	A	A	1
az14701HidalgoCoNM	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	A	A	1
az14705HdalgoCoNM	**ariz**	**AxC**	**G**	**G**	**T**	**A**	**C**	**T**	**T**	**A/T**	**C/G**	**A**	**A**	**2**
az14716HidalgoCoNM	**ariz**	**AxC**	**A/G**	**G**	**T**	**A**	**C**	**A/T**	**T**	**A/T**	**C/G**	**A**	**A**	**4**
coa14827HuecoTanks	ariz	coah	G	G	T	A	C	T	T	T	**C/G**	A	A	2
coa14828HuecoTanks	**ariz**	**AxC**	**G**	**G**	**T**	**A**	**C**	**T**	**T**	**A/T**	**C**	**A**	**A**	**1**
coa14829HuecoTanks	**ariz**	**AxC**	**A/G**	**G**	**C/T**	**A**	**C**	**T**	**C/T**	**A/T**	**C/G**	**A**	**A/G**	**6**
coa14830HuecoTanks	**ariz**	**AxC**	**A/G**	**G**	**C/T**	**A**	**C**	**T**	**C/T**	**A/T**	**C/G**	**A**	**A/G**	**6**
coa14831HuecoTanks	**ariz**	**AxC**	**A/G**	**G**	**C/T**	**A**	**C**	**A/T**	**C/T**	**A/T**	**C/G**	**A**	**A/G**	**7**
coa14832HuecoTanks	ariz	coah	G	G	T	A	C	T	T	A	**C/G**	A	A	1
coa14833HuecoTanks	**ariz**	**AxC**	**G**	**G**	**T**	**A**	**C**	**T**	**T**	**A/T**	**C/G**	**A**	**A**	**2**
coa14834HuecoTanks	ariz	ariz	G	G	T	A	C	T	T	A	G	A	A	0
coa14835HuecoTanks	ariz	ariz	G	G	T	A	C	T	T	A	G	A	A	0
coa14798 Quitman Mtns.	**ariz**	**AxC**	**A/G**	**G**	**T**	**A**	**C**	**A/T**	**T**	**A/T**	**C**	**A**	**A**	**3**
coa14799 Quitman Mtns.	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	A	A	1
coa14800 Quitman Mtns.	ariz	ariz	G	G	T	A	C	T	T	A	C	A	A	0
coa14801 Quitman Mtns.	**ariz**	**AxC**	**G**	**G**	**T**	**A**	**C**	**T**	**T**	**A/T**	**C**	**A**	**A**	**1**
coa14802 Quitman Mtns.	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	**A/C**	A	2
coa14803QuitmanMtns.804R	**ariz**	**AxC**	**G**	**G**	**T**	**A**	**C**	**T**	**T**	**A/T**	**C/G**	**A/C**	**A**	**3**
coa14804Quitman Mtns. 804R	ariz	coah	G	G	T	A	C	T	T	T	C	A	A	0
coa14805Quitman Mtns.	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	**A/C**	A	2
coa14806Quitman Mtns.	ariz	ariz	G	G	T	A	C	T	T	A	**C/G**	**A/C**	A	2
coa14807sofAlpine	coah	coah	G	G	del	**A/G**	**C/T**	**C/T**	**C/T**	T	C	A	A	4
coa14808sofAlpine	coah	coah	G	G	T	A	C	T	**C/T**	T	C	A	A	1
coa14810sofAlpine	coah	coah	G	G	del	A	**C/T**	**C/T**	T	T	C	A	A	2
coa14811sofAlpine	coah	coah	G	A	del	**A/G**	**C/T**	T	T	T	C	A	A	2
coa14812wofAlpine	coah	coah	G	G	del	**A/G**	**C/T**	T	**C/T**	T	C	A	A	3
coa14813wofAlpine 313R	coah	coah	G	G	T	A	**C/T**	T	**C/T**	T	C	A	A	2
coa14814wofAlpine	coah	coah	G	G	T	A	C	T	T	T	C	A	A	0
coa14815wofAlpine	coah	coah	G	G	del	**A/G**	**C/T**	T	**C/T**	T	C	A	A	3
coa14816wofAlpine	coah	coah	G	G	del	A	**C/T**	T	**C/T**	T	C	A	A	2
coa14817FtDavis 1000Y	coah	coah	G	G	T	A	C	T	**C/T**	T	C	A	A	1
coa14818FtDavis	coah	coah	G	G	del	A	T	T	T	T	C	A	A	0
coa14819FtDavis	coah	coah	G	G	del	G	T	T	T	T	C	A	A	0
coa14820FtDavis 689K	coah	coah	G	**A/G**	del	**A/G**	**C/T**	T	T	T	C	A	A	3
coa14821FtDavis 1100Y	coah	coah	G	G	del	**A/G**	T	T	T	T	C	A	A	1
coa14822sofMarfa	coah	coah	G	**A/G**	T	A	C	T	T	T	C	A	A	1
coa14823sofMarfa1100Y	coah	coah	G	G	T	A	C	T	T	T	C	A	A	0
coa14824sofMarfa	coah	coah	G	G	del	**A/G**	T	T	T	T	C	A	A	1
coa14825sofMarfa	**coah**	**AxC**	**G**	**A/G**	**T**	**A**	**C**	**T**	**T**	**A/T**	**C**	**A**	**A**	**2**
coa14826sofMarfaY1100	coah	coah	G	**A/G**	del	na	na	na	na	T	C	A	A	1
coa6829LaZarca	coah	coah	G	**A/G**	T	A	C	T	T	T	C	A	A	1
coa6830LaZarca	coah	coah	G	A	T	A	C	T	T	T	C	A	A	0
coa6831LaZarca	coah	coah	G	**A/G**	T	A	C	T	T	T	C	A	A	**1**
coa10241km45nDgo	coah	coah	G	G	T	A	C	T	T	T	**C/T**	A	A	**1**
coa10242km45nDgo,503Y	coah	coah	G	G	T	A	C	T	T	T	**C/T**	A	A	**1**
number of polymorphic			**5**	**6**	**3**	**7**	**8**	**5**	**10**	**11**	**24**	**4**	**3**	